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HOST ENHANCEMENT OF SYMBIONT PHOTOSYNTHESIS IN THE HYDRA-ALGAE SYMBIOSIS

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Abstract

Photosynthesis by the *Chlorella*-like algal symbiont of the green hydra, *Hydra viridis*, was determined for algae *in situ* and *in vitro* by measuring photosynthetic oxygen evolution in a modified polarographic electrode. Effects of light intensity, environmental oxygen concentration, and bicarbonate ion concentration on photosynthetic oxygen evolution were measured, with the following results: (1) Bicarbonate ion increased photosynthesis by algal symbionts *in situ* with up to 1 m*M* bicarbonate added. (2) Based on light intensity/photosynthesis data, photosynthetic oxygen evolution in symbionts *in situ* was greater than in those *in vitro*, especially at ambient oxygen concentrations. Oxygen severely inhibited photosynthetic oxygen evolution by symbionts *in vitro* but had little or no effect on algae *in situ* up to ambient oxygen concentrations. These data suggest that hydra symbionts gain a significant photosynthetic advantage, especially at ambient (=8 mg l^{-1}) oxygen concentrations, when they are associated with their hosts. The role of the host-symbiont relationship in contributing to this advantage is discussed.

Introduction

The green hydra, *Hydra viridis*, harbors *Chlorella*-like symbionts within its gastrodermal cells. These photosynthetically active algae provide the host with photosynthetically fixed carbon, mainly in the form of maltose (Muscatine and Lenhoff, 1963; Muscatine, 1965), which augments the hydra's nutrition under starvation conditions (Muscatine and Lenhoff, 1965; Pardy and White, 1977). The nutritional advantage of the symbiosis to the host is clear. The advantages of the association to the algae are not so evident.

Oxygen inhibition of photosynthesis in *Chlorella* was first described by Warburg (1920). Oxygen may inhibit photosynthesis by oxidizing photosynthetic components, by decreasing reducing equivalents needed for carbon fixation, and by competing with CO_2 at the carboxylating enzyme, ribulose 1,5 bisphosphate carboxylase/oxygenase. This last mechanism leads to the phenomenon known as photorespiration, the light stimulated uptake of O_2 and release of CO_2 (Goldsworthy, 1970; Chollet and Ogren, 1975; Tolbert, 1979; Zelitch, 1979). Photorespiration does not conserve net energy and results in a net loss of carbon.

Downton *et al.* (1976) determined that increasing oxygen concentration substantially inhibited oxygen evolution by *Tridacna* symbionts *in vitro*, but presented no data on the effects of oxygen on symbionts *in situ*. If oxygen also inhibits photosynthesis in hydra symbionts, algae within the host might realize a photo-

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Abbreviations: 3-(3-4-dichlorophenyl)-1,1-dimethylurea, DCMU; rate of photosynthesis at light saturation, P_{max}; light intensity at onset of light saturation, I_k; respiratory quotient, R.Q.; substrate concentration at which rate of enzymatic reaction is half maximal, K_m; photosynthetically active radiation, PAR.

synthetic advantage. Decreased oxygen and increased CO_2, conditions expected within respiring host tissues, would tend to favor photosynthesis. We examined hydra algae *in situ* and *in vitro* to determine the magnitude of O_2 inhibition of photosynthesis, and the effects of light and exogenous CO_2 on photosynthetic oxygen evolution. *In vitro* experiments required isolation of symbionts from their host and examination of their photosynthetic performance in an artificial medium. Such an approach has proved fruitful in previous studies of symbiotic associations such as in the release of maltose by hydra symbionts (Muscatine, 1965), the release of photosynthate from zooxanthellae (Trench, 1971a) and the effects of host homogenate on the release of glycerol by zooxanthellae (Trench, 1971b). Such analysis always risks the introduction of effects specific to the artificial environment devised for the symbionts, but as yet no worker has been able to deduce or duplicate exactly the conditions occurring within any host organism, so this risk is true of any system used.

Materials and Methods

Experimental organisms

Green hydra (*Hydra viridis*, Florida strain) were mass-cultured in M solution and fed daily with brine shrimp (*Artemia salina*) nauplii, as described by Lenhoff and Brown (1970), except that the M solution contained no bicarbonate. Cultures were maintained at 20°C in a photoperiod incubator (Freas 818, Precision Scientific Co.), set for constant illumination by a single 40-watt cool-white fluorescent tube (Sylvania F48T10). Quantum flux at the center of the incubator was 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR) as measured with a Licor LI-170 quantum sensor (Lambda Instruments). Animals were not fed for 24 h before they were used in experiments.

Oxygen measurements

A slide projector with a 500-watt lamp (Sylvania DAY-DAK Tungsten) and infrared filter provided light for photosynthesis experiments (Fig. 1). A series of mirrors directed the projector beam to a collimating lens. This lens focused light onto the surface of a Plexiglas light guide inserted into the chamber of a Rank Brothers oxygen electrode (Rank Brothers, Cambridge, England). Light intensity was regulated using neutral density filters (Bausch and Lomb) for coarse adjustment and a diaphragm for fine adjustment. Light intensity at the base of the light guide was measured using a Licor quantum sensor. The spectral emission of the projector lamp was analyzed using an Isco scanning spectroradiometer (Model SR, Instrumentation Specialties Co.) (Fig. 2). The O_2 concentration in the chamber was recorded continuously by connecting the electrode to a chart recorder (Fisher Recordall 5000, Fisher Scientific Co.). The chamber was maintained at 20°C with a circulating, temperature-controlled water bath (Markline 2095, Forma Scientific Co.).

Sodium dithionite ($\simeq$5 mg) added to 5 ml of distilled water in the electrode chamber calibrated the electrode at zero O_2 concentration. An IBC dissolved oxygen meter (Model 500-051, International Biophysics Inc.) was used to determine the O_2 concentration in air-saturated M solution ($\simeq$8.4 mg O_2 l^{-1}) which was used to set the span of the electrode.

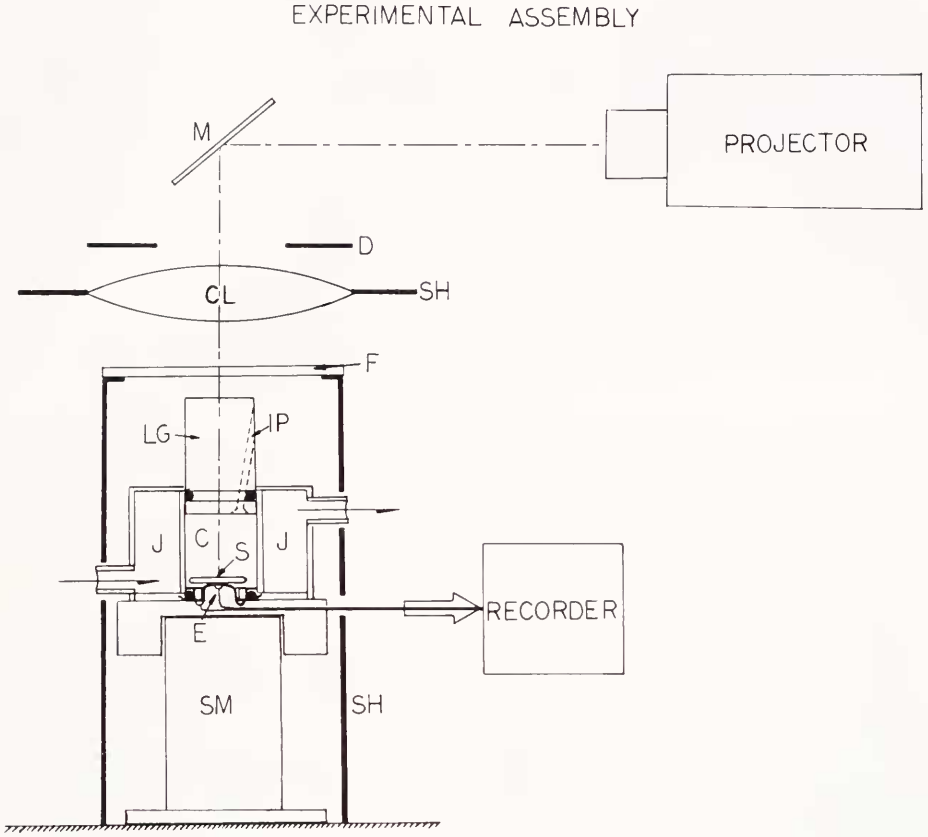

FIGURE 1. Apparatus used to measure photosynthetic oxygen evolution. M = mirror assembly; CL = collimating lens; D = diaphragm; SH = stray light shield; F = neutral density filter(s); LG = light guide; IP = injection port; C = sample chamber; S = stir bar; J = water jacket; E = polarographic oxygen electrode; SM = stirring motor.

Photosynthesis of H. viridis *symbionts* in situ

About 150 hydra were used to determine O_2 evolution by algal symbionts *in situ*. Carryover of contaminants with the animals was minimized by washing them with fresh M solution before they were placed in the oxygen electrode chamber. Animals were placed in a small basket and suspended in 5 ml of fresh M solution (pH 7.5). $KHCO_3$ was added to a final concentration of 1.0 m*M* in all experiments with intact hydra except those designed to test the effect of this compound on symbiont photosynthesis (our M solution contained about 0.140 m*M* HCO_3^- from atmospheric CO_2). The addition of $KHCO_3$ assured that the symbionts were not CO_2 limited during illumination at higher light intensities. Oxygen evolution was determined over a 5–10 min period and the rate of oxygen evolution determined from these data. Light intensity-photosynthesis curves were constructed by varying the light intensity impinging on the chamber as previously described with 1.0 m*M* $KHCO_3$ present in the chamber. The effects of $KHCO_3$ on O_2 evolution were determined by varying this parameter at a fixed light intensity, and the effects of

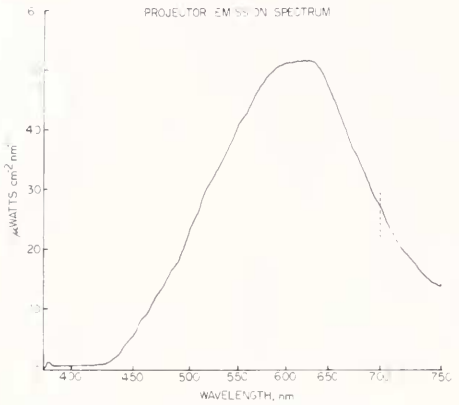

FIGURE 2. Emission spectrum of projector lamp as determined with a scanning spectroradiometer. Dotted lines define range of photosynthetically active radiation (P.A.R.).

oxygen on O_2 evolution were determined by varying O_2 concentration at 1 m*M* $KHCO_3$ and a fixed light intensity.

Photosynthesis of H. viridis *symbionts* in vitro

Symbionts for *in vitro* studies were isolated from hosts by homogenizing approximately 150 hydra using a Potter Elvenjeim tissue homogenizer with a Teflon pestle in 100 m*M* potassium phosphate buffer (pH 6.4) containing 1 m*M* $MgSO_4$ and 1 m*M* $CaCl_2$. Algae were pelleted from the homogenate using a tabletop centrifuge at $1700 \times g$ for 1 min. The algal pellet was washed 6 times and finally resuspended in 5 ml of homogenization buffer. Buffer was added to yield a concentration of approximately 2×10^7 algal symbionts in a 5 ml sample. This concentration yielded maximum photosynthesis by the isolated symbionts as determined in this laboratory (data not shown). A 5 ml aliquot was placed in the electrode chamber, and oxygen concentration was adjusted by bubbling with nitrogen. $KHCO_3$ added to the chamber to a final concentration of 12 m*M* yielded sufficient CO_2 to prevent CO_2 limitation at higher light intensities. The electrode chamber was sealed with the Plexiglas light guide and measurements of oxygen evolved were made for 5–10 min in the light. Rates of oxygen evolution were determined from these data.

Chlorophyll content

Following O_2 measurements on intact hydras, animals were homogenized as described previously. Total chlorophyll (Chl) content of symbionts both *in situ* and *in vitro* was assayed using a 10 μl sample of suspension extracted in 3 ml of absolute methanol. Fluorescence of the methanolic extracts was measured in a Turner III Fluorimeter (Turner and Associates) using a 5-60 primary filter and a 2-64 secondary filter (Turner and Associates). The fluorimeter was calibrated using chlorophyll standards prepared from methanolic extracts of symbionts and analyzed for total chlorophyll as described by Mackinney (1941), using a Spectronic-20 spectrophotometer. The 150 hydra used in experiments yielded about $1.5–3.0 \times 10^{-2}$ mg chlorophyll and algal suspensions were adjusted to yield 1.0×10^{-2} mg Chl.

Rates of oxygen evolution

Rates of O_2 evolution for symbionts *in situ* were determined by subtracting oxygen consumption in the dark from O_2 evolution in the light. For algal suspensions, dark respiration was not subtracted from rates observed in the light. In light, dark respiration is suppressed in algae (Brown and Tregunna, 1967). Furthermore, respiration in *Chlorella* has been shown to be less than 10% of the rate of photosynthesis (Brown and Tregunna, 1967), and we have found that symbionts *in vitro* also exhibit rather low rates of dark respiration. These rates were so close to zero at 1–2 mg O_2 l^{-1} that they were not accurately measurable with our apparatus for the most part. The *Hydra viridis* respiration rate averaged about 0.53 μmoles $min^{-1} \cdot mg\ chl^{-1}$, much greater than that observed for the algae alone. We therefore felt justified in ignoring the respiratory component of the algae in measuring oxygen evolution by whole animals.

Results

The effects of exogenous bicarbonate on photosynthesis by hydra symbionts in situ

Adding HCO_3^- ions to the medium strongly affected photosynthetic oxygen evolution by *in situ* symbionts of *H. viridis* (Fig. 3). Oxygen evolution by the symbionts increased sharply with up to 1 m*M* added bicarbonate, followed by a much slower increase on further additions.

The effects of incident light intensity on photosynthesis by hydra symbionts

Photosynthetic O_2 evolution by *H. viridis* symbionts *in situ* and *in vitro* increased with increasing incident light intensity (Fig. 4, 5). However, photosynthesis by the symbionts was dramatically influenced by the O_2 concentration of the medium and whether the symbionts were *in situ* or *in vitro*. At O_2 concentrations ranging from 0.5–1.5 mg O_2 l^{-1} (Fig. 4) rates of O_2 evolution *in vitro* were only slightly lower than those *in situ*. In contrast, at an O_2 concentration of 6.5–7.0 mg l^{-1} (Fig. 5) rates of oxygen evolution by symbionts *in vitro* were much lower than those of symbionts *in situ* at all light intensities examined.

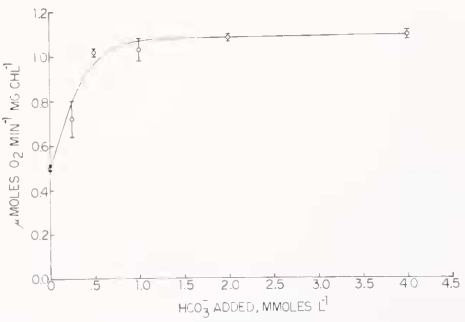

FIGURE 3. The effect of added bicarbonate on oxygen evolution by *H. viridis* algal symbionts *in situ*. $P_{max} = 11.9 \times 10^{-4}$ mmoles O_2 min^{-1} mg Chl^{-1}. Hydra were incubated in M solution, pH 7.5, which was purged with N_2 to an oxygen concentration of 1.5–2.0 mg O_2 l^{-1}. Circles = mean values. Vertical bars = S.D. n = 2 to 4. Each point = integrated rate of O_2 evolution over 5–10 min period.

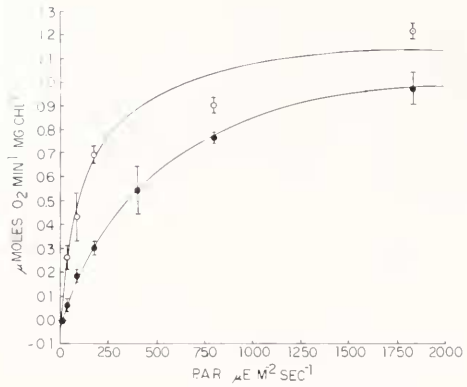

FIGURE 4. Oxygen evolution as a function of light intensity by symbionts of *H. viridis* at low (0.5–1.5 mg l^{-1}) oxygen concentrations. Open circles = algal symbionts *in situ*, hydra maintained in M solution + 1 m*M* $KHCO_3$. Closed circles = algal symbionts *in vitro* in phosphate buffer, pH 6.4 + 12 m*M* $KHCO_3$. Circles = mean values. Vertical bars = S.D. n = 2 to 4. Each point = integrated rate of O_2 evolution over 5–10 min period.

The light intensity-photosynthesis curves were analyzed on the basis of: the rate of photosynthesis at light saturation, P_{max} (maximal rate of photosynthesis); the light intensity at the onset of light saturation, I_k (Talling, 1957; Yentsch and Lee, 1966); and the slope of the light-limited portion of the curves (Yentsch and Lee, 1966). At O_2 concentrations near ambient (6.5–7.0 mg l^{-1}) the P_{max} of symbionts *in vitro* was almost 95% lower than that of those *in situ* (Table 1). In addition, the slope of the curves in the light-limited region decreased substantially for symbionts *in vitro* compared to those *in situ*, increasing I_k about twofold in isolated algae. In contrast, at lower O_2 concentrations (0.5–1.5 mg l^{-1}) symbionts *in vitro* had a slightly lower P_{max} (15%) than those *in situ*, while the slope of the curve during light limitation decreased about the same as at near ambient O_2 concentrations. This increased I_k about fourfold.

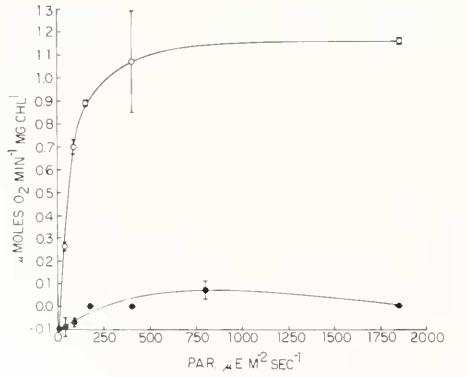

FIGURE 5. Oxygen evolution as a function of light intensity by symbionts of *H. viridis* at near ambient (6.5–7.0 mg l^{-1}) oxygen concentrations. Open circles = algal symbionts *in situ*, hydra maintained in M solution + 1 m*M* $KHCO_3$. Closed circles = algal symbionts *in vitro* in phosphate buffer, pH 6.4 + 12 m*M* $KHCO_3$. Circles = mean values. Vertical bars = S.D. n = 2 to 4. Each point = integrated rate of O_2 evolution over 5–10 min period.

TABLE I

Kinetics of oxygen evolution as a function of light intensity for algae of H. viridis. *Slope is that of initial region of curves in Figures 4 and 5.*

O_2 Conc.	Symbiont location	P_{max} mM O_2 min^{-1} mg chl^{-1}	I_k μE m^{-2} s^1	Slope
6.5–7.0 mg O_2 l^{-1}	*in situ*	1.16	125	13
	in vitro	0.06	280	0.67
0.5–1.0 mg O_2 l^{-1}	*in situ*	1.13	125	13
	in vitro	0.96	520	2.4

Thus, photosynthetic response to light decreased in algal symbionts isolated from their hosts, and this decrease was greater at higher O_2 concentrations. In contrast, the photosynthetic response to light of symbionts *in situ* was relatively insensitive to oxygen.

Oxygen inhibition of photosynthetic oxygen evolution

As the light intensity-photosynthesis data suggested, whether the symbionts were *in situ* or *in vitro* strongly influenced the inhibition of photosynthetic oxygen evolution by O_2 (Fig. 6). Increasing environmental O_2 concentration from low to near air-saturation strongly inhibited O_2 evolution by symbionts *in vitro*. In contrast, symbionts *in situ* were only slightly affected, and only at the higher O_2 concentrations. This is evident from percent inhibition of O_2 evolution as a function of O_2 concentration (Fig. 7). For example, at 8 mg O_2 l^{-1} symbionts *in vitro* were inhibited 110%, but symbionts *in situ* only 11%.

DISCUSSION

The Rank Brothers polargraphic oxygen electrode has been used to study oxygen evolution by a coral and its symbiotic zooxanthellae (Crossland and Barnes, 1977) and by cell suspensions of photosynthetic organisms (Findenegg, 1976; Burris, 1977; Takabe and Akazawa, 1977). Our use of the Plexiglas light guide avoids the len-

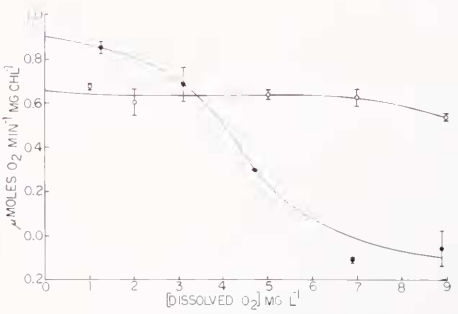

FIGURE 6. Oxygen evolution by *H. viridis* symbionts as a function of oxygen concentration. Open circles = algal symbionts *in situ*, hydra incubated at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ in M solution + 1 mM $KHCO_3$. Closed circles = algal symbionts *in vitro* in phosphate buffer, pH 6.4 + 12 mM $KHCO_3$ at 600 $\mu E \cdot m^{-2} \cdot s^{-1}$. Initial oxygen concentrations were produced by N_2 purging. Circles = mean values. Vertical bars = S.D. n = 2 to 4. Each point = integrated rate of O_2 evolution over 5–10 min period.

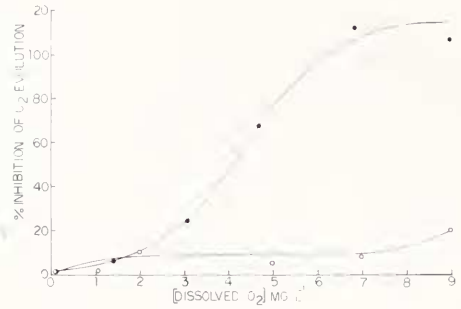

FIGURE 7. Percent inhibition of *H. viridis* symbiont oxygen evolution by oxygen. Open circles = algal symbionts *in situ*. Closed circles = algal symbionts *in vitro*. Each point represents means of Figures 5 and 6.

ticular effects of the electrode's cylindrical jacket and chamber walls, which otherwise increase or decrease the light actually reaching organisms in the chamber. The light guide evenly illuminates small animals such as hydra. Finally, with our modifications, light intensity measurements are easier, requiring only a 2π light sensor.

Symbionts were isolated in phosphate buffer to avoid organic buffering systems the symbiotic algae might metabolize. For instance, *Chlorella pyrenoidosa* will photometabolize acetate (Goulding and Merrett, 1966). Other workers have used phosphate-citrate buffer with algal symbionts (Cernichiari *et al.*, 1969) but have presented no data concerning its possible metabolism. The ion concentrations used in our buffer were derived empirically; the pH was adjusted to 6.4 because this was found to give the maximum oxygen evolution at a given light intensity. This pH also yields roughly equal concentrations of CO_2 and HCO_3^- in solution.

Symbionts *in situ* carried out a basal rate of photosynthetic O_2 evolution without added exogenous bicarbonate. Under these conditions the association consumed O_2 at a rate of 0.53 μmol $O_2 \cdot min^{-1} \cdot mg\ chl^{-1}$, which we attributed to host respiration. The R.Q., defined as the ratio of the CO_2 produced to O_2 consumed by an organism (Richardson, 1929; Kleiber, 1961), may be used to calculate the CO_2 production of an organism if its O_2 consumption is known. Pardy and White (1977) calculated the R.Q. for hydra unfed for 24 h at 0.862 at ambient O_2 concentrations. This value gives an estimated CO_2 output of 0.46 μmol $CO_2 \cdot min^{-1} \cdot mg\ chl^{-1}$. Assuming that CO_2 fixed into carbohydrates is the main sink for reducing power generated by photolysis of water, a mole of O_2 should be evolved from the symbionts for every mole of CO_2 taken up. Thus, under conditions where the sole source of CO_2 for symbiont photosynthesis is the host respiration, oxygen evolution by the symbionts *in situ* should be about 0.46 μmol $O_2 \cdot min^{-1} \cdot mg\ chl^{-1}$. The slight discrepancy between this value and the observed O_2 evolution of 0.50 μmol $O_2 \cdot min^{-1} \cdot mg\ chl^{-1}$ may be attributed to O_2 evolution by symbionts due to non-CO_2 fixing electron flow (Anderson, 1978) or to the small amount of CO_2 remaining in the M solution after purging with N_2.

Thus, with little or no CO_2 available externally, the rate of photosynthesis of hydra symbionts depends on the rate at which the host generates CO_2 internally, provided the association is not light-limited. Under such conditions the symbiotic association will not show net oxygen production, as an R.Q. greater than 1 would

be required of host metabolism. Pardy and Dieckmann (1975) probably were unable to show net O_2 evolution in *H. viridis* for this reason. Reisser (1980) reached a similar conclusion.

However, when bicarbonate is available in the medium, algal symbionts are able to use the added CO_2 to photosynthesize. They are mainly limited by other factors, such as light intensity, and not by host CO_2 production. This is shown by the 120% increase we observed with the addition of as little as 1 m*M* $KHCO_3$. Reisser (1980) reported similar increases for the *Paramecium/Chlorella* symbiotic association upon additions of bicarbonate. Net O_2 evolution from the *Hydra/Chlorella* symbiotic association occurred under these conditions. We have observed light compensation for the intact association as low as 175 $\mu E \cdot m^{-2} \cdot s^{-1}$, a little less than one-tenth full sunlight. Above this value the association produces oxygen rather than consumes it. Thus, in ecological terms, when light and CO_2 are abundant, the green hydra symbiotic association becomes a producer. The hydra is, in effect, a carnivorous plant.

Summarizing, when the environmental concentration of CO_2 is low, photosynthesis by hydra symbionts depends on, and is therefore modulated by, host CO_2 evolution at moderate light intensities. In contrast, when environmental CO_2 is abundant, the rate of photosynthesis is limited by other factors, such as light.

Rates of photosynthetic O_2 evolution for hydra symbionts *in situ* were generally lower than those for other symbiotic or free-living *Chlorella*. Webb *et al.* (1980) reported a rate of oxygen evolution for *Chlorella vulgaris* five times the values that we observed for *H. viridis* symbionts measured *in situ*. Similarly, Reisser (1980) reported a rate of O_2 evolution for *Paramecium* symbionts *in situ* about six times the values we obtained for hydra symbionts. Some of these differences could be due to culture and incubation conditions: for example, Pardy and White (1977) showed that fed green hydras have respiration rates threefold higher than unfed controls.

Determinations of photosynthetic O_2 evolution as a function of incident light intensity have been reported for corals and their zooxanthellae (Wethey and Porter, 1976; Crossland and Barnes, 1977). Wethey and Porter (1976) used Michaelis-Menten kinetics to analyze their light intensity-photosynthesis data, claiming that the "K_m" (substrate concentration at half maximal rates of an enzymatic reaction) they obtained corresponded to the "affinity" of photosynthesizing organisms for light.

We analyzed our photosynthetic light response curves on the basis of the maximum rate of photosynthesis, P_{max}; the light intensity at the onset of P_{max}, I_k (Talling, 1957; Yentsch and Lee, 1966), and the initial slope of the light intensity-photosynthesis curve. Changes in the initial slope may be related to changes in the rate of the light reactions at a nonsaturating light intensity, while changes in P_{max} may be ascribed to changes in the overall rate of the CO_2 fixing reactions of photosynthesis (Yentsch and Lee, 1966).

Our data show that symbiont photosynthesis is profoundly affected by both the association with the animal host and the O_2 concentration in the medium. Removing symbionts from their hosts decreases the slope in the light-limited region of the light response curve, which is relatively independent of the O_2 concentration. This effect does not seem to be due to a difference in light-harvesting ability of suspensions of algae and intact hydra due to mutual shading. If this were the case, the more dilute cell suspension should exhibit a steeper slope than that of symbionts *in situ*, as the symbionts *in vitro* are less densely concentrated in the electrode

chamber than when they are within the hydra. However, symbionts *in situ* exhibit the steeper slope.

As the oxygen concentration increases from low to near ambient, both the P_{max} and the I_k of symbionts *in vitro* decrease. Such changes have been taken as indicative of a photosynthetic response to an inferior environment (Yentsch and Lee, 1966). In this case the inferior environment is the more highly oxygenated one. In contrast, P_{max} and I_k of symbionts *in situ* change very little or not at all between low and near ambient O_2 concentrations, indicating no change in photosynthetic response over the range of O_2 concentrations examined.

The lower P_{max} of symbionts *in vitro* than that *in situ* indicates inhibition of the CO_2-fixing reactions of photosynthesis. Although this decrease is evident at both O_2 concentrations examined, it is greatest at the higher one. This implies that oxygen (and to a lesser extent the removal of the symbionts from their hosts), acting directly or indirectly on the CO_2-fixing reactions of photosynthesis, inhibits O_2 evolution at light saturation.

Oxygen may inhibit photosynthetic O_2 evolution due to photooxidative damage of the light-harvesting and photosystem pigments, or to pseudocyclic electron flow. Both of these mechanisms have been implicated at relatively high O_2 concentrations in *Chlorella pyrenoidosa* (Shelp and Canvin, 1980). Either mechanism might affect the Calvin cycle by decreasing the reducing equivalents available for CO_2 fixation, thus accounting for the decrease in P_{max}. Oxygen also competes with CO_2 at the active site of the carboxylating enzyme, ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase, resulting in the oxygenase function of the enzyme (Chollet and Ogren, 1975; Tolbert, 1979). This reaction produces glycolate, the substrate of photorespiration in higher plants and algae (Goldsworthy, 1970; Chollet and Ogren, 1975; Beck, 1979; Tolbert, 1979). In algae, oxygen reacts at two loci in the photorespiratory pathway: at the RuBP carboxylate/oxygenase and in the conversion of glycine to serine. Hence, the apparent rate of photosynthetic oxygen evolution by algae is reduced with increasing O_2 concentrations. However, at the HCO_3^- concentrations we employed this probably would be minor.

The inhibiting effects of O_2 were markedly less in those algae associated with the host than those *in vitro*. The higher P_{max} in symbionts *in situ* than in symbionts *in vitro* shows that at near ambient O_2 concentrations the maximum photosynthetic capacity of symbionts *in vitro* was drastically less than that of symbionts *in situ*. Furthermore, symbionts *in vitro* were more inhibited by O_2 than those *in situ* when photosynthetic O_2 evolution was examined over a wide range of O_2 concentrations and at light intensities which negated the effects of isolation from the host.

Based on data collected so far a model may be postulated: the close proximity between host and symbiont may lead to a cycling of carbon and O_2 that favors symbiont photosynthesis. Possibly the hydra mitochondrial CO_2 production raises the CO_2 concentration near the algal symbionts, as has been postulated to occur in *Paramecium* (Reisser, 1980). Moreover, host mitochondrial activity can lower the O_2 concentration near the symbionts, which would decrease the inhibitory effects of O_2.

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